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(Vorsteher: Prof. Dr. R. Schuppli)

Virus Antigen Antibody Reactions by Gel Diffusion

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Melvin Paul Hennisch

von New York



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S. KARGER

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Virus Antigen Antibody Reactions by Gel Diffusion

By M. P. HENNISCH

From the Department of Allergology of the Dermatological Clinic, University of Basel
(Head: Prof. R. SCHUPPLI)

The usual methods for testing the presence of viral antibodies are complement fixation, precipitin reaction and haemagglutination inhibition. The aim of this paper is to test the applicability of the antigen antibody reaction of viruses by means of gel diffusion using the micro OUCHTERLONY method, i.e. to determine if there is a specific diagnostic precipitate for each virus or viral strain.

Materials and Methods

Buffer Solution. An isotonic SÖRENSEN disodium phosphate-citric acid buffer, pH 7,0, containing Merthiolate 1:10,000, was used as the solvent for all solids and as the diluent for all solutions.

Agar. A 1,5% agar gel was prepared by pressure sterilizing, at 120°C for 30 minutes, a mixture of 1,5 grams of agar per 100 ccm. of buffer. The agar was then filtered hot, collected, and stored at 1°C till used.

Micro Method. The experiments were conducted by the micro OUCHTERLONY method which has several advantages over the macro method, namely the quantities of reactants used, the micro method requires only 1/100 the quantity, and the time necessary for results to visualize, the micro method requires only 24–36 hours as opposed to the macro method which requires 6–12 days.

Microscopic slides, 26 mm × 76 mm, were uniformly covered with 2 ccm. of agar. By means of a metal template, seven basins, equidistant from one another, 6 mm., were punched out of the agar with a 15 gauge hypodermic needle, 1,5 mm. diameter. The

center basin contained antibody and the outer basins antigen. The basins are numbered clockwise with 1 at 12 o'clock and were always filled to the brim. After 24–36 hours of diffusion the slides were prepared for staining. They were first washed in distilled water for 3 hours and then covered with blotting paper and dried. When completely dried, the blotting paper was removed and the paper fragments washed away. The slides were stained 5 minutes in an amidoschwarz solution containing 1.0 gram amidoschwarz to 1 liter of a 9:1 methanol-glacial acetic solution. The slides were destained in a 9:1 methanol-glacial acetic acid solution about 3 minutes, then washed with distilled water and air dried.

In all experiments the same buffer and agar concentrations were used and all experiments were conducted in duplicate at room temperature, 18–22°C.

Antigen and Antibody

Different influenza virus strains were used as antigen*. Rabbit serum was employed as the antibody source, the rabbit being inoculated with A Singapore strain. The haemagglutination inhibition titer was 1:2048. The antiserum was tested for reaction with chicken allantoic fluid and was found to be negative. All basins were filled twice using undiluted serum and virus culture. Three different arrangements of basins were used:

1. three basin system (figs. 1–6);
2. seven basin system with central basin containing allantoic fluid (figs. 7–10);
3. seven basin system with central basin containing antiserum (figs. 11 and 12).

<i>Strain</i>	<i>Haemagglutination Titer</i>
A Japan	1:160
A Singapore	1:1028
A'FM ₁	1:1028
B Lee	1:2056

Results and Conclusions

The results show that influenza virus gives a specific precipitate pattern with its homologous antiserum. The same two antigens were present in A'FM₁ (A-prime virus), main cause of influenza

* All antigens and antibodies were put at our disposal by the Schweiz. Serum- und Impfinstitut, Bern.

1946-1957, but in reverse concentrations as indicated by the dissymmetry and crossing over of the precipitates (fig. 1). This is in agreement with the theory that the suppression of the dominant antigen of a virus to that of a secondary antigen and the new dominance of a secondary antigen is brought about by an in-

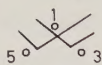


Fig. 1

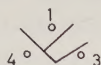


Fig. 2

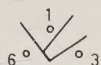


Fig. 3

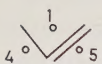


Fig. 4

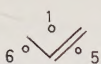


Fig. 5

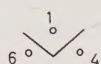


Fig. 6

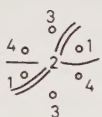


Fig. 7

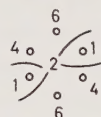


Fig. 8

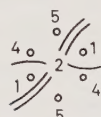


Fig. 9

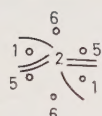


Fig. 10

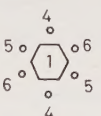


Fig. 11



Fig. 12

Figs. 1-6. Three basin system.

Figs. 7-10. Seven basin system with allantoic fluid in central basin.

Figs. 11-12. Seven basin system with rabbit antiserum in central basin.

1 = Rabbit antiserum.

4 = A Japan.

2 = Allantoic fluid

5 = A'FM₁

3 = A Singapore

6 = B Lee.

creasing antibody titer in the general population, thereby hindering the propagation of the virus. In support of this theory MULDER AND MASUREL have shown the presence of antibodies to the Asian strains of 1957 in people above the age of 40 years, particularly in those people over the age of 70.

The results of A Singapore antiserum and A Japan (Asian strain 1957) and B Lee virus show the similarity of influenza virus

strains to one another (figs. 2-6). These two strains show the presence of a polyvalent antigen, i.e. a protein complex containing different prosthetic groups each capable of reacting with its specific antibody independent of the other, reacting with both of the antibodies demonstrated in A Singapore. This polyvalent antigen may be a result of the virus undergoing mutation, for it is currently believed that the nucleoprotein antigen carries the genetic determination of the virus. This antigen, present in A Japan and B Lee, is identical in both strains.

The preliminary experiment conducted clearly calls for the further investigation of viral antigen antibody reactions by means of gel diffusion. A prerequisite for this investigation would be the utilization of haemagglutination and haemagglutination inhibition titers in the magnitude of 100,000 or greater in order that all antigens present may be clearly demonstrated, as it has been proven that the critical concentration for each antigen antibody reaction is different. RHODE AND KÜHN, by means of the Oudin double diffusion technic of gel diffusion, have shown antigenic differences in vaccinia preparations from different sources due to culture medium.

The advantage of gel diffusion over the other methods is that one may directly compare the antigenic composition of one virus strain to another. Gel diffusion offers a new diagnostic procedure which will prove invaluable in epidemiology and the production of standard purified vaccines and toxoids.

Summary

Using the micro OUCHTERLONY method of gel diffusion, a series of experiments have been conducted to determine the relationship of different influenza virus strains to one another. The results clearly show the similarity of different A strains to one another by the presence of identical antigens and the similarity of A strains to B strains by the presence of similar or identical antigens.

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Author's address: Dr. M. P. Hennisch, 3760-88th Street, Jackson Heights 72, N. Y. (USA).



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